Effect of Pancreas Transplantation and Immunosuppression on Proinsulin Secretion

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Insulin resistance and increased demand for insulin secretion occur after successful pancreas transplantation. To investigate the potential effects of immunosuppression and pancreas transplantation on fasting β-cell function, we studied fasting proinsulin and 32,33 split proinsulin secretion cross-sectionally and longitudinally in segmental pancreatic graft recipients (SPx, *n* = 18); in whole-pancreas graft recipients (WPx, *n* = 13); in nondiabetic kidney transplant recipients (Kx, *n* = 14) and in normal subjects (Ns, *n* = 14). Basal insulin secretion rates were significantly increased in SPx 15.8 (1.7), WPx 24.4 (4.5) and Kx 22.1 (2.1) vs Ns 9.7 (1.6) pmol min^{-1} l^{-1}, $p < 0.05$, mean (SEM). Total proinsulin, intact proinsulin and 32,33 split proinsulin concentrations were significantly higher in all the transplanted groups than in normal subjects ($p < 0.05$), whereas the total proinsulin to C-peptide ratio and the 32,33 split proinsulin ratio were higher in SPx than in WPx, Kx and Ns (< 0.05). In the longitudinal study, β-cell function in terms of proinsulin secretion remained stable for 1 year. In conclusion, fasting glucose homeostasis in pancreas-kidney transplant recipients is obtained at the expense of increased proinsulin secretion and increased insulin secretion rates, primarily induced by immunosuppression. In segmental pancreas graft recipients, increased fasting proinsulin and 32,33 split proinsulin relative to the number of β-cells transplanted indicate more stress on the residual β-cell and therefore higher secretory demand than in whole pancreas transplant recipients. © 1998 John Wiley & Sons, Ltd.

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Introduction

Several studies of pancreas graft recipients have shown abnormalities in β-cell function following stimulation with β-cell secretagogues, despite normal levels of fasting plasma glucose and glycosylated haemoglobin (HbA_{1c}).[1–6] A probable explanation is that the reduced number of β-cells in the pancreas graft are unable adequately to enhance insulin secretion in response to the immunosuppressive induced insulin resistance.[1–6] A direct effect of the immunosuppressive treatment on insulin synthesis and release has also been suggested.[5,7]

In plasma from healthy people, approximately 90 % of proinsulin-like molecules consists of intact proinsulin and des 31,32 proinsulin, the remainder being composed of the other three split products including 32,33 split proinsulin.[8,9] Increased secretion of proinsulin, and in some cases also of proinsulin split products, either absolute or relative to insulin or C-peptide levels, has been described in various diabetic or diabetes-related states,[10–14] during short-term dexamethasone-induced insulin resistance in healthy people[15,16] and in hemi-pancreatectomized donors.[17] A similar picture predicts the transition from impaired glucose tolerance to Type 2 diabetes mellitus in Japanese Americans.[18] It has been proposed that hyperproinsulinaemia could represent a very early marker of β-cell dysfunction or injury.[19] The mechanism of this disproportionate hyperproinsulinaemia is not clear. One obvious explanation is that the increased demand on the β-cells stresses them, resulting in premature release of immature insulin secretory granules with a high content of proinsulin. Alternatively it could be a manifestation of a defect in proinsulin processing in the β-cells.[20] We have previously shown, using an assay that did not distinguish between the intact form of proinsulin and the split products, that the total proinsulin

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secretion is absolutely and relatively increased in segmental pancreas transplant recipients.[4]

In the present study, we wished to extend our previous evaluation of the fasting β-cell function in segmental pancreas transplant recipients[4] by assessing intact proinsulin and 32,33 split proinsulin levels in a group of successful pancreas transplants receiving a segmental and a group receiving a whole pancreatic graft and determine whether these parameters indicate β-cell dysfunction. A second aim of the investigation was to take into account the effect of immunosuppressive treatment on the insulin secretory apparatus. Therefore, two control groups were included: (1) nondiabetic kidney transplant recipients treated with similar immunosuppression requirements and (2) nondiabetic healthy controls. Lastly, to investigate whether proinsulin and 32,33 split proinsulin secretion remain stable over time, we also obtained longitudinal data over 1 year.

Patients and methods

Cross-sectional Study

Informed consent was obtained from four groups of subjects. We recruited one group of 18 successful segmental pancreas–kidney transplant recipients (SPx) and one group of 13 successful whole pancreas–kidney transplant recipients (WPx), both groups with systemic delivery of insulin. The segmental pancreas transplants had either enteric (n = 14) or urinary drainage of the exocrine secretion (n = 4). In addition, 14 nondiabetic kidney transplant recipients with portal insulin secretion from their native pancreas were recruited as the first control group (Kx). All the transplanted groups had received cadaveric grafts used and the immunosuppressive medication consisted of prednisolone 5–10 mg day^{-1}, cyclosporine A 150–300 mg day^{-1} and azathioprine 50–75 mg day^{-1}. The transplanted subjects were studied 40 (5) months (SPx), 28 (4) months (WPx), and 32 (5) months (Kx) after transplantation (mean (SEM), $p < 0.05$). Fourteen nondiabetic controls served as the second control group (Ns). Normal levels of fasting glucose and HbA_{1C} (nondiabetic range 4.1 %–6.1 %) were required for participation in the study. All subjects were studied as outpatients consuming an *ad libitum* diet, including 300 g of carbohydrate for at least 3 days prior to testing.

Longitudinal Study

To determine whether there had been any changes in fasting β-cell parameters over time, a subgroup of the cross-sectional participants was restudied. Ten SPx recipients, seven WPx recipients, six Kx recipients, and eight healthy subjects were re-studied, approximately 1 year after the initial evaluation. During this time, there had been no signs of rejection episodes among the transplanted subjects and no changes had been made in the immunosuppressive treatment. The studies, approved by the local Ethics Committees, were performed in accordance with the Helsinki Declaration.

Study Design and Assay Methods

After an overnight fast of 10 h, venous blood samples were obtained for determinations of the levels of plasma glucose, C-peptide, serum insulin, proinsulin, 32,33 split proinsulin, glycosylated haemoglobin (HbA_{1C}), cyclosporine A. Weight and height were recorded and body mass index (BMI) calculated (kg m^{-2}). Plasma glucose was determined in duplicate by a glucose oxidase method (Beckman Instruments, Fullerton, CA, USA). Blood samples for determinations of plasma C-peptide, serum insulin and proinsulin levels were centrifuged immediately at 3000 RPM for 10 min at 4 °C and stored at −20 °C pending analyses. Serum insulin was measured by two-site IRMA, in which there is no cross-reactivity with proinsulin and 32,33 split proinsulin.[8] Intact proinsulin and 32,33 split proinsulin were measured by two-site IRMA.[8] In this last assay, des-32 split proinsulin and des-31,32 split proinsulin are also measured and the 32,33 split proinsulin levels are corrected for cross-reactivity of intact proinsulin (84 %).[8,21] We use the term 32,33 split proinsulin to indicate the sum of these three molecules and most of them are des-31,32 split proinsulin.[8,21] The sensitivity of the intact proinsulin and 32,33 split proinsulin assays was 1.25 pmol l^{-1}. Total serum proinsulin was measured using a two-site monoclonal ELISA technique.[22] After removal of proinsulin by PEG precipitation, C-peptide was determined by radioimmunoassay in duplicate, using antiserum M1221 (Novo Nordisk A/S, Copenhagen, Denmark).[23] Glycosylated haemoglobin (HbA_{1C}) was analysed by a HPLC method. The samples from each pancreas–kidney transplant recipient were measured with samples from a control subject in the same run.

Since basal insulin secretion is difficult to evaluate using basal C-peptide or insulin levels after pancreas transplantation,[2,4] basal insulin secretion rates were calculated based on the concomitantly measured serum insulin and C-peptide by the 'combined model' of insulin and C-peptide kinetics, which takes account of the altered kinetics of insulin and C-peptide in the transplanted groups.[24] Basal insulin secretion rates were correlated to total proinsulin, intact proinsulin, and 32,33 split proinsulin.

Statistical Methods

The data from the two pancreas transplanted groups were compared statistically to Kx and Ns and the Kx data were compared to the Ns data. Non-parametric statistical tests were employed: the Mann-Whitney rank sum test in the analyses of unpaired data, and the Wilcoxon rank sum test in the analyses of paired data. All results in the text and tables are presented as the mean (SEM). The level of significance was set at $p < 0.05$.

Table 1. Clinical characteristics of the subjects in the cross-sectional study; segmental pancreas transplant recipients (SPx), whole pancreas transplant recipients (WPx), kidney transplant recipients (Kx), and normal subjects (Ns)

	SPx	WPx	Kx	Ns
Number F/M	18 (7/11)	13 (4/9)	14 (6/8)	14 (6/8)
Age (years)	43 (1)	41 (2)	43 (2)	43 (2)
Body mass index (kg m^{-2})	23.5 (0.9)	22.6 (0.6)	23.5 (0.7)	23.7 (0.6)
Plasma glucose (mmol l^{-1})	4.8 (0.1)	4.7 (0.2)	5.0 (0.2)	4.9 (0.1)
HbA_{1C} (%)	5.2 (0.1)	5.3 (0.2)	5.5 (0.1)	5.3 (0.1)
Serum creatinine (mmol l^{-1})	121 (9)[a c]	151 (12)[a]	130 (7)[a]	80 (3)
Cyclosporine dosage (mg day^{-1})	224 (13)	241 (18)	267 (27)	–
Plasma cyclosporine (ng ml^{-1})	195 (30)[b]	219 (26)[b]	309 (23)	–
Prednisone dosage (mg day^{-1})	7.5 (0.5)	8.0 (0.4)	7.0 (0.3)	–

Mean (SEM).
[a] $p < 0.05$ compared to Ns; [b] $p < 0.05$ compared to Kx; [c] $p < 0.05$ compared to WPx.

Results

Cross-sectional Study

The clinical characteristics of the participating subjects at baseline are given in Table 1. Body mass index, fasting plasma glucose concentrations, and glycosylated haemoglobin A_{1C} levels were similar in all groups, but serum creatinine levels were higher in the pancreas–kidney transplanted groups (SPx and WPx) and in the nondiabetic kidney transplanted group (Kx) than in the healthy subjects (Ns) ($p < 0.05$). The plasma cyclosporine levels were significantly higher in Kx than in the two pancreas transplanted groups.

Table 2 depicts the β-cell-related hormone results. The insulin data demonstrate the effect of peripheral insulin delivery and decreased first-pass hepatic insulin extraction in the pancreas transplanted groups, with higher levels in these groups than in Kx and Ns. In contrast, C-peptide levels were higher in Kx than in SPx and Ns, but similar to WPx (Table 2). Absolute total proinsulin was significantly higher in the three transplanted groups than in Ns, but was not different between the transplanted groups. Intact proinsulin concentrations were also higher in the transplanted groups, compared to Ns, and within the transplanted groups intact proinsulin was significantly higher in Kx than in SPx. The 32,33 split proinsulin levels were significantly higher in Kx than in the other groups, but SPx and WPx also had higher levels than Ns.

The results of the calculation of ratios are demonstrated in Table 2 and Figure 1. The total proinsulin to insulin ratio was significantly decreased in the two pancreas transplanted groups, compared to Kx and Ns. The intact proinsulin to insulin ratio was significantly higher in Kx than in the other groups. The 32,33 split proinsulin to insulin ratio was higher in Kx than in SPx, WPx and Ns. The ratio was lower in WPx compared with Ns, whereas no difference was found between SPx and Ns.

Total proinsulin to C-peptide and 32,33 split proinsulin to C-peptide ratio were higher in SPx than in the other groups (Table 2), whereas the intact proinsulin to C-peptide ratios were not significantly different between the groups. The serum insulin to C-peptide ratios were

Table 2. Cross-sectional findings in the participating subjects; fasting plasma C-peptide, serum insulin, proinsulin and 32,33 split proinsulin in segmental pancreas transplant recipients (SPx, $n = 18$), whole pancreas transplant recipients (WPx, $n = 13$), kidney transplant recipients (Kx, $n = 14$) and normal subjects (Ns, $n = 14$)

	SPx	WPx	Kx	Ns
Serum insulin (pmol l^{-1})	126 (17)[a]	142 (12)[a]	93 (20)[a]	42 (6)
Plasma C-peptide (pmol l^{-1})	877 (120)[a,b,c]	1391 (190)[a]	1572 (130)[a]	494 (40)
Serum total proinsulin (pmol l^{-1})	22.7 (4.2)[a]	21.5 (4.7)[a]	28.1 (8.3)[a]	10.6 (1.1)
Serum intact proinsulin (pmol l^{-1})	6.7 (1.4)[a,b]	9.0 (1.1)[a]	13.5 (3.3)[a]	3.0 (0.3)
Serum 32,33 split proinsulin (pmol l^{-1})	15.2 (3.2)[a,b]	11.6 (1.7)[a,b]	27.6 (7.4)[a]	5.9 (0.5)
Total proinsulin/insulin ratio	17.4 (2.2)[a,b]	14.7 (2.8)[a,b]	28.7 (5.1)	29.5 (3.3)
Intact proinsulin/insulin ratio	6.0 (1.1)[a,b]	6.7 (0.9)	18.6 (4.7)[a,c]	9.2 (1.1)
32,33 split proinsulin/insulin ratio	14.5 (4.2)[b]	8.2 (1.2)[a,b]	37.3 (9.1)[a]	19.3 (3.3)
Total proinsulin/C-peptide ratio	24.8 (5.0)[a,b,c]	15.1 (2.1)	14.3 (2.9)	18.1 (2.5)
Intact proinsulin/C-peptide ratio	7.6 (0.9)	7.8 (1.5)	8.0 (1.5)	6.5 (0.5)
32–33 split proinsulin/C-peptide ratio	20.2 (3.2)[b]	8.6 (2.4)[a]	16.3 (4.1)	13.5 (2.3)
Insulin/C-peptide ratio	15.1 (1.1)[a,b]	12.2 (2.1)[a,b]	6.1 (0.9)[a]	9.2 (1.1)
Basal insulin secretion rate (pmol min^{-1} l^{-1})	15.8 (1.7)[a]	24.4 (4.5)[a]	22.1 (2.1)[a]	9.7 (1.6)

Mean (SEM).
[a] $p < 0.05$ compared to Ns; [b] $p < 0.05$ compared to Kx; [c] $p < 0.05$ compared to WPx.

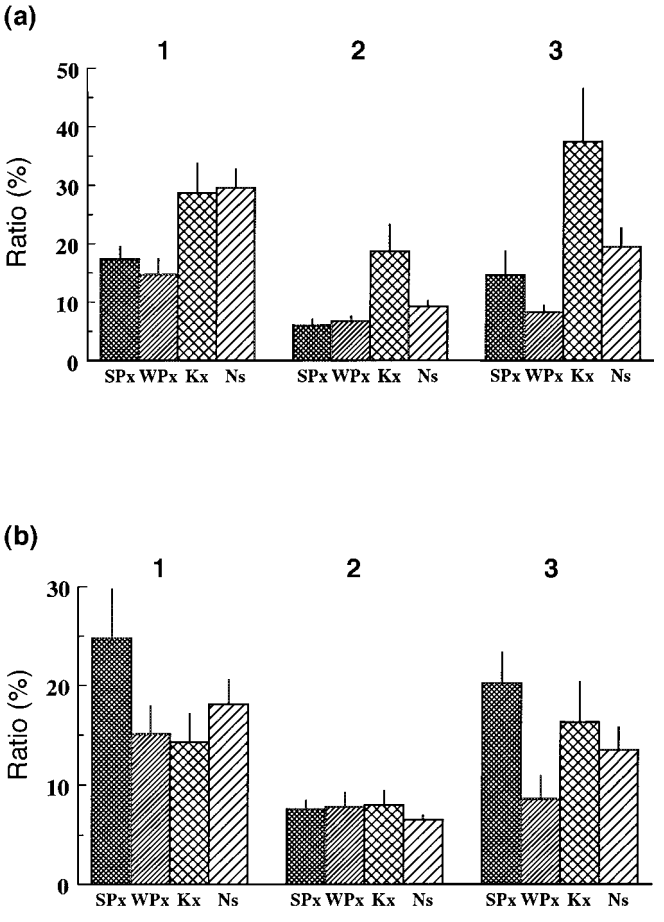

Figure 1. (a) Total proinsulin/insulin (1), intact proinsulin/insulin (2) and 32,33 split proinsulin/insulin (3) ratios, and (b) total proinsulin/C-peptide (1), intact proinsulin/C-peptide (2) and 32–33 split proinsulin/C-peptide (3) ratio in segmental pancreas transplant recipients (SPx), whole pancreas transplant recipients (WPx), kidney transplant recipients (Kx) and normal subjects (Ns). Mean (SEM). For exact figure and statistical evaluation see Table 2

increased in the pancreas transplanted groups, but not in Kx and Ns, and Kx had a lower ratio than Ns.

Basal insulin secretion rates were significantly higher in all the transplanted groups than in healthy subjects (Table 2). Basal insulin secretion rates tended to be lower in SPx than in WPx and Kx, but there were no significant differences between the groups. Although a negative tendency was found in the relation between basal insulin secretion rates and total proinsulin and intact proinsulin or 32,33 split proinsulin, no significant correlations were found in any of the groups. The total proinsulin to basal insulin secretion rate tended to be higher in SPx than in the other groups, but no significant differences were observed in total proinsulin, intact proinsulin or 32,33 split proinsulin to the basal insulin secretion rate in any of the groups (data not shown).

Longitudinal Study

A subgroup of participants in the longitudinal study, consisting of 10 SPx recipients, 7 WPx recipients, 6 Kx recipients, and 8 normal subjects, was restudied after a period of 12.7 (3.1), 10.1 (2.5), 9.3 (2.3), 13.0 (2.1) months, respectively (p = NS). The ages were not significantly different in these groups, SPx 44 (1), WPx 42 (2), Kx 44 (2) and Ns 44 (2) years. Glycosylated haemoglobin A_{1C} levels were also not different in the groups and had not changed significantly during the study period: SPx 5.5 (0.1)/5.6 (0.2), WPx 5.7 (0.1)/5.6 (0.2), Kx 5.6 (0.2)/5.5 (0.1), Ns 5.1 (0.1)/5.2 (0.1) %, respectively. Kidney function, evaluated by the serum creatinine levels, remained stable throughout the study and was significantly higher in WPx and Kx than in SPx and Ns, and in SPx than in Ns, SPx 109 (8)/112 (7), WPx 144 (10)/134 (8), Kx 155 (8)/145 (9), and Ns 81 (3)/85 (4) μmol l^{-1}, respectively. The immunosuppressive treatment (prednisone, cyclosporine A, azathioprine) remained unchanged during the study period and did not differ from the data given in the cross-sectional study. The body mass index also remained stable during the study period (SPx 23.3 (0.3)/23.0 (0.5), WPx 22.3 (0.3)/22.4 (0.4), Kx 23.5 (0.4)/23.6 (0.8), Ns 23.4 (0.3)/23.3 (0.5) kg m^{-2}, respectively).

The β-cell variables are shown in Table 3. Fasting plasma glucose was stable in all groups during the study period. Likewise, fasting serum insulin was higher in the pancreas transplanted groups than in Kx and Ns, and it remained higher. In contrast, fasting plasma C-peptide was significantly higher in WPx and Kx than in SPx and Ns at both sampling times. Although plasma C-peptide declined slightly over time in SPx, it remained higher than in Ns ($p < 0.05$). Intact proinsulin was significantly higher in the transplanted groups at the two sampling times and did not change in the groups. Similarly, 32,33 split proinsulin level was significantly higher in the transplanted groups than in Ns, and it remained higher at follow-up.

Calculation of the individual ratios gave the same pattern as for the cross-sectional data and none of the calculated ratios changed significantly during the study period (Table 3). The 32,33 split proinsulin to C-peptide ratio was higher in SPx than in WPx, Kx and Ns. The intact proinsulin to C-peptide ratios were not significantly different between the groups. The intact proinsulin to insulin ratio was significantly higher in Kx than in SPx, WPx and Ns.

Discussion

In the present study, the effect of immunosuppression and of the type of pancreas transplantation (segmental versus whole-pancreas allografts) on basal β-cell secretion was assessed in a cross-sectional and longitudinal study design, to determine whether proinsulin and/or 32,33 split proinsulin secretion could be used as an index of β-cell dysfunction. This could be of advantage if such measurements could be used instead of more time-consuming testing, such as oral glucose tolerance or intravenous glucose tolerance tests. Since the pancreas

Table 3. Clinical characteristics and β-cell variables at baseline (A) and at follow-up (B) in the longitudinal study of segmental pancreas transplant recipients (SPx), whole pancreas transplant recipients (WPx), kidney transplant recipients (Kx) and normal subjects (Ns)

	SPx	WPx	Kx	Ns
Number (F/M)	10 (5/5)	7 (1/6)	6 (1/5)	8 (3/5)
Plasma glucose (mmol l^{-1})				
A	4.9 (0.3)	4.9 (0.3)	5.1 (0.2)	4.7 (0.2)
B	4.9 (0.2)	4.6 (0.2)	5.1 (0.2)	4.6 (0.2)
Plasma C-peptide (pmol l^{-1})				
A	807 (160)[a,b,c]	1305 (242)[a]	1662 (282)[a]	513 (46)
B	663 (71)[a,b,c]	1232 (171)[a]	1279 (182)[a]	442 (27)
Serum insulin (pmol l^{-1})				
A	122 (23)[a]	150 (19)[a]	98 (24)[a]	32 (4)
B	140 (25)[a]	174 (24)[a]	82 (18)[a]	39 (5)
Serum intact proinsulin (pmol l^{-1})				
A	7.2 (2.1)[a]	9.2 (1.7)[a]	13.7 (4.2)[a]	3.2 (0.4)
B	7.4 (2.4)[a]	8.8 (1.9)[a]	9.0 (1.7)[a]	3.1 (0.4)
Serum 32,33 split-proinsulin (pmol l^{-1})				
A	16.2 (5.5)[a]	12.4 (2.9)[a]	33.0 (7.8)[a]	5.9 (0.9)
B	19.4 (7.8)[a]	14.0 (2.1)[a]	16.8 (3.8)[a]	6.1 (1.2)
Intact proinsulin/insulin ratio				
A	5.9 (1.0)[a,b]	6.1 (0.9)[b]	13.9 (2.6)[a]	9.9 (0.9)
B	5.3 (0.8)[a,b]	5.0 (0.7)[b]	11.0 (2.4)[a]	7.9 (0.6)
Intact proinsulin/C-peptide ratio				
A	8.9 (0.9)	7.0 (1.2)	8.2 (1.2)	6.2 (0.4)
B	11.0 (1.1)	7.1 (1.1)	7.0 (1.1)	7.0 (0.3)
32,33 split proinsulin/C-peptide ratio				
A	20.0 (3.1)[a,c]	9.5 (2.1)	15.8 (3.9)	11.5 (2.3)
B	22.2 (3.3)[a,c]	11.3 (2.0)	13.1 (3.7)	13.8 (2.4)
Insulin/C-peptide ratio				
A	15.1 (1.2)[a,b]	11.4 (2.0)[a,b]	5.9 (0.7)[a]	6.2 (0.8)
B	21.1 (1.5)[a,b]	14.1 (2.2)[a,b]	6.4 (0.8)[a]	8.8 (1.1)

Mean (SEM).
[a] $p < 0.05$ compared to Ns; [b] $p < 0.05$ compared to Kx; [c] $p < 0.05$ compared to WPx.

transplant recipients and the control group of nondiabetic kidney transplant recipients received identical immunosuppressive therapy, the data also provide insights into whether the immunosuppressive treatment results in an inappropriate secretion of proinsulin products. The importance of an effect of different demands relative to the number of β-cells for proinsulin secretion was investigated by choosing a group of segmental and a group of whole pancreas transplanted recipients.

The two pancreas transplanted groups had hyperinsulinaemia, partly caused by the peripheral insulin secretion without first-pass hepatic insulin extraction, and partly caused by the immunosuppression inducing insulin resistance, as previously reported.[1–7,25] Denervation of the pancreas might also potentially contribute to the hyperinsulinaemia. Thus, defects in the negative feedback of insulin on insulin secretion has been shown during euglycemic hyperinsulinaemia and during hypoglycaemia.[26,27] The hypersecretion of insulin in the transplanted groups is demonstrated by calculated insulin secretion rates, which were higher than in healthy subjects. The relatively high C-peptide levels in transplanted recipients however may partially be related to the reduced kidney function and thereby decreased clearance of C-peptide.[2,4]

Mature secretory granules in the β-cells are thought to contain a small fixed amount of proinsulin. Thus, in general, in the basal state proinsulin and insulin or C-peptide concentrations are in equilibrium and, as the β-cell increases or reduces its secretion of insulin, it might be expected also to increase or reduce secretion of proinsulin in a proportionate fashion. Yet our two pancreas-transplanted groups had a higher secretion of total proinsulin, intact proinsulin and 32,33 split proinsulin than the healthy subjects. The two pancreas-transplanted groups did not differ in these parameters, indicating that the β-cells in the segmental pancreas grafts secreted the same amount of insulin precursors as did the β-cells in the whole-organ pancreas grafts. Furthermore, the levels of proinsulin and the split products were not different between the kidney- and pancreas-transplanted recipients.

Few data are available about the proinsulin secretion and synthesis during long-term immunosuppression and

in recipients of a pancreas transplant without exogenous insulin administration. Osei *et al*. reported normal proinsulin levels in whole-pancreas transplant recipients;[3] however, the levels during fasting glycaemia are not clear from their paper.[3] We have previously demonstrated increased total proinsulin secretion in segmental pancreas transplant recipients in the fasting state which was further enhanced following meal stimulation, both absolutely and relatively to C-peptide concentrations.[4]

To evaluate whether the higher proinsulin concentration may be an index of β-cell dysfunction, the proinsulin concentration should be related to the amount of insulin secreted. As a substitute, the proinsulin concentrations have been related to the concomitant insulin concentration.[11–15] We found lower total proinsulin, intact proinsulin, and 32,33 split proinsulin to insulin ratios in the two pancreas transplanted groups than in normal subjects and kidney transplanted recipients. This is most obviously explained by the loss of first-pass hepatic insulin extraction in the pancreas transplanted recipients.

The total proinsulin/C-peptide and 32,33 split proinsulin/C-peptide ratios were higher in segmental pancreas transplanted recipients than in the other two transplanted groups. The intact proinsulin/C-peptide ratios were not different between the four groups. Since the actual C-peptide levels overestimate the insulin secretion rates by 40–50 % in transplanted recipients, the results indicate an inappropriately high secretion of total proinsulin and 32,33 split proinsulin in all the transplanted groups, most pronounced in the segmental transplant recipients.[4] The changes in C-peptide kinetics were approximately the same in the three transplanted groups.[4] When the total proinsulin, intact proinsulin and 32,33 split proinsulin levels were related to the estimated insulin secretion rate, only the total proinsulin/insulin secretion rate was somewhat higher in the segmental pancreas transplanted recipients.

Two main hypotheses have been suggested to account for inappropriate hypersecretion of proinsulin.[19,20] First, an increased demand on insulin secretion depletes the β-cell of mature insulin granules, resulting in a population of less mature and proinsulin-rich granules for secretion ('overworked β-cell' hypothesis). It is likely that the insulin resistance and especially the reduced number of β-cells in the segmental transplant recipients would require an increased insulin secretion by each β-cell and to explain the present data. Our findings are consistent with the findings of the absolute and relative hyperproinsulinaemia in hemipancreatectomized donors and data from Leavy *et al*. in 90 % pancreatectomized rats.[17,28] Alternatively, an intrinsic defect in the enzymatic proinsulin processing mechanism has been proposed.[20] Whether such defects in proinsulin processing are induced by the immunosuppressive agents used after pancreas and kidney transplantation is not clear, but glucocorticoids have been known to increase the proinsulin-to-insulin ratio.[29] In accordance with the last hypothesis is the inappropriate hyperproinsulinaemia in the kidney transplanted recipients when compared with normal subjects.

Direct measurements of the kinetics of proinsulin and split products and dose–relation between proinsulin, intact proinsulin, and split proinsulin with increasing serum creatinine have not been reported from renal transplant recipients. Clearance of these peptides is mainly mediated by the kidney and proinsulin levels are elevated in chronic renal failure.[30,31] Decreased clearance of proinsulin might be expected in the transplanted subjects because of their reduced kidney function. The disproportionately high proinsulin and split product levels in the kidney transplanted group compared to normal subjects could also be explained by a decreased clearance of proinsulin. At present, no methods can simultaneously adjust for the reduced clearance of C-peptide and proinsulin and split proinsulin.

The present study also provided evidence that several years after transplantation, β-cell function was stable during a 1-year period without changes in the immunosuppressive treatment and without clincal episodes of rejection. These data may argue against the 'overworked β-cell' hypothesis and a longer follow up might give different results. However, other studies have also shown stable β-cell function over time after pancreas transplantation.[32–34]

The biological activity and physiological significance of the high proinsulin and split products are not fully understood. In relation to glucose metabolism, proinsulin is approximately 10 % as biologically active as that of insulin but acts preferentially on the liver rather than on the peripheral tissues in man.[35] The levels of proinsulin and split products in the present study, and in a previous study, were not high enough to indicate any biological effect on glucose homeostasis as seen in the study by Davis *et al*.[4,35]

An undesirable influence of increased levels of proinsulin and 32,33 split proinsulin on atherogenesis and cardiovascular events has been suggested in some but not all studies.[36–38] Whether this is also true in pancreas transplant recipients is not known.

In summary, the pancreas–kidney transplant recipients and kidney transplant recipients demonstrated increased peripheral concentrations of proinsulin and proinsulin split products. These changes may be explained by insulin resistance induced by the immunosuppressive therapy, leading to a secondarily increased secretory demand on the β-cells and the effect *per se* of the immunosuppressive treatment on the insulin secretory apparatus, with a partial contribution from reduced clearance of these peptides. The increased fasting proinsulin and 32,33 split proinsulin relative to the number of β-cells transplanted in segmental pancreas-transplant recipients indicate a higher secretory demand in these recipients. β-Cell function in terms of proinsulin secretion seems to remain stable over time.

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